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A Fast Transient Outward Monovalent Current in Rat Saphenous Myocytes Passing Through Ca^{2+} Channels

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Abstract. Transient outward currents in rat saphenous arterial myocytes were studied using the perforated configuration of the patch-clamp method. When myocytes were bathed in a Na-gluconate solution containing TEA to block large-conductance Ca^{2+}-activated K^+ (BK) currents, depolarizing pulses positive to +20 mV from a holding potential of −100 mV induced fast transient outward currents. The activation and inactivation time constants of the current were voltage dependent, and at +40 mV were 3.6 ± 0.8 ms and 23.9 ± 6.4 ms (n = 4), respectively. The steady-state inactivation of the transient outward current was steeply voltage dependent (z = 1.7), with 50% of the current inactivated at −55 mV. The current was insensitive to the A-type K^+ channel blocker 4-AP (1–5 mM), and was modulated by external Ca, decreasing to approximately 0.85 of control values upon raising Ca^{2+} from 1 to 10 mM, and increasing approximately 3-fold upon lowering it to 0.1 mM. Transient outward currents were also recorded following replacement of internal K^+ with either Na^+ or Cs^+, raising the possibility that the current was carried by monovalent ions passing through voltage-gated Ca^{2+} channels. This hypothesis was supported by the finding that the transient outward current had the same inactivation rate as the inward Ba^{2+} current, and that both currents were effectively blocked by the L-type Ca^{2+} channel blocker, nifedipine and enhanced by the agonist BAYK8644.

Key words: Arterial smooth muscle — Patch clamp — Transient outward current — Calcium channel

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Introduction

Transient outward K^+ currents have been reported in many vascular smooth muscle cells. The kinetics and voltage dependence of their activation and inactivation, as well as their pharmacological profile appear to be tissue dependent. In several smooth muscle cell types, transient K^+ currents have a low activation threshold, near −40 mV, and fast activation and inactivation kinetics with a time to peak and inactivation time constant lower than 10 and 50 ms, respectively. The voltage dependence of (steady-state) inactivation is very steep, with midpoints typically in the range of −70 and −90 mV, falling almost to zero at about −50 mV (Beech & Bolton, 1989; Clapp & Gurney, 1991; James, Okada & Horie, 1995; Yuan et al., 1998). Current amplitude and reversal potential measurements in ion substitution experiments showed that the fast transient outward K^+ current was highly selective for K^+ ions, with an unresolvable permeability to Na^+ and Cs^+ (James et al., 1995). The currents were insensitive to TEA, but selectively blocked by 4-AP, with a half-inhibitory concentration in the range of 0.6–2 mM (Beech & Bolton, 1989; Smirnov & Aaronson, 1992; Yuan et al., 1998; but *see* also James et al., 1995). In general, these properties were consistent with those of the cardiac transient outward currents and the A-currents first described in neurons (Connor & Stevens, 1971; Hagiwara, Yoshida & Yoshii, 1981), and subsequently found in many excitable cells.

Our study on rat saphenous artery myocytes (using the perforated-patch whole-cell recording technique and conditions that eliminate the normally dominant large-conductance Ca^{2+}-activated K^+ current; c.f. Catacuzzeno et al., 2000), revealed a transient outward current. This current had kinetic properties, and voltage activation and inactivation characteristics similar to the fast transient outward

K^+ currents described in several types of vascular smooth muscle cells. The transient current was, however, insensitive to 4-AP, but inhibited by nifedipine and enhanced by BAYK8644. Moreover, it was poorly selective for K^+; Na^+ and Cs^+ can also be charge carriers. Finally, the current amplitude was dependent on external Ca, being markedly enhanced upon lowering the Ca^{2+} concentration to the micromolar range. These features are inconsistent with those previously reported for fast transient outward K^+ currents in vascular smooth muscle cells. They are, however, congruent with monovalent cation currents flowing through Ca^{2+} channels, previously reported in numerous preparations (Reuter & Scholz, 1977; Fenwick, Marty & Neher, 1982; Lee & Tsien, 1982; Almers, McCleskey & Palade, 1984; Fukushima & Hagiwara, 1985; Lang, 1990).

Voltage-gated Ca^{2+} channels, which under normal conditions are highly selective, can become permeant to monovalent cations when the Ca^{2+} concentration on the side where the ion flux originates is very low (≤10 μM). Studying skeletal muscle fibres, Almers and coworkers (1984a and b) found that when the external Ca^{2+} is reduced, the inward Ca^{2+} current falls proportionally, reaching a minimal (undetectable) current between 1 mM and 10 μM. However, upon further decreasing the external Ca^{2+}, the inward current increased again. It was convincingly demonstrated that under these conditions, the inward current was carried by external Na^+ (or any other extracellular monovalent cation). Monovalent cations can also carry currents through Ca^{2+} channels in the outward direction, in some cases even when the external Ca^{2+} is in the millimolar range. The first evidence for this behavior was provided by Reuter & Scholz (1977) for cardiac muscle, later confirmed by Lee & Tsien (1982). Subsequently, several other groups have established that monovalent cations can carry outward currents through Ca^{2+} channels in a variety of tissues, even in the presence of significant external Ca^{2+} (Fenwick et al., 1982; Almers et al., 1984; Fukushima & Hagiwara, 1985). Based on these findings, we set out to test the hypothesis that the fast transient outward current we recorded on perforated patches from rat saphenous artery myocytes was due to monovalent cations flowing through Ca^{2+} channels. We present biophysical and pharmacological results supporting this notion.

Materials and Methods

Myocyte Isolation Procedure

Male Wistar rats (weight range, 150–175 g) from Charles River-Italia (Como, Italy) were used. The animals were killed by concussion and cervical dislocation, and the saphenous arteries were quickly removed. They were split open longitudinally and bathed in ice-cold low-Ca^{2+} physiological saline solution (in mM: NaCl 140, KCl 5, $CaCl_2$ 0.16, $MgCl_2$ 2, MOPS 5, glucose 10), and carefully cleared of adhering fat and connective tissue. The arteries were then transferred and kept overnight at 4°C in the isolating medium (in mM: NaCl 110, KCl 5, $CaCl_2$ 0.16, $MgCl_2$ 2, MOPS 10, K_2HPO_4 0.5, Na_2HPO_4 0.5, glucose 10, EDTA 0.49, taurine 10) containing in addition papain 0.03% (Sigma, St. Louis, MO), collagenase 0.024% (Worthington Biochemical) and BSA 0.2% (Sigma). The proteolytic reaction was initiated by adding 1 mM dithiothreitol (Sigma) and maintaining the arteries at 37°C under gentle agitation for 15 min. Myocytes were dissociated and dispersed by trituration. The isolation procedure produced good yields of relaxed saphenous smooth muscle cells ca. 125 × 6 μm (average length × width). The isolated saphenous myocytes contracted when superfused with the α_1 agonist phenylephrine and remained viable for several hours when maintained at room temperature. Only spindle-shaped, relaxed cells were used for electrophysiological experiments, performed 3 to 9 hours after isolation.

Electrophysiology

Macroscopic currents were recorded using the perforated-patch method (Horn & Marty, 1988), which allows the exchange of monovalent, but not divalent ions, between the pipette and the cell. Electrical access to the cytoplasm was achieved by adding amphotericin B (0.5 μM). Access resistances in the range 10–15 MΩ were achieved within 15 min after seal formation. Experiments began following complete exchange of the internal K^+ with the monovalent cation in the pipette, normally 10 minutes after the cell access resistance became stable. This requirement was considered to be satisfied when the amplitude of the current evoked by a train of voltage pulses (0.1 Hz) stabilized. Before recording the macroscopic currents, at least 60% of the series resistance and capacitance were compensated. Leakage current was estimated by hyperpolarizing pulses and, in some instances, subtracted (*see* Figure legends). Borosilicate pipettes (Hilgenberg, Malsfeld, Germany), pulled with a programmable puller (PUL-100; WPI, Sarasota, FL, USA) were used. Their resistances ranged between 3 and 6 MΩ when filled with standard internal solutions. Currents were amplified with a List EPC-7 amplifier (List Medical Instruments, Darmstadt, Germany), and digitized with a 12-bit A/D converter (TL-1, DMA interface; Axon Instruments, Union City, CA). The pClamp software package (version 5.6; Axon Instruments) was used on a Compaq Pentium PC for generating the command voltage pulses, recording and archiving the currents, and preliminary analysis of the data. For on-line data collection, current signals were normally filtered at 10 kHz and sampled at 50 kHz.

Solutions

The superperfusing solution used to isolate the transient outward currents contained (in mM): Na-gluconate 140, K-gluconate 5, $CaCl_2$ 1, $MgCl_2$ 5, MOPS 5, TEA 10, glucose 10 (pH 7.2). The superfusing solution used for inward Ba current recordings contained (in mM): $BaCl_2$ 90, MOPS 5, glucose 10 (pH 7.2). The pipette solution contained (in mM): 90 KCl, 30 K_2SO_4, 2 $MgCl_2$, 10 MOPS, pH 7.2. Amphotericin B was added at a concentration of 0.5 μM to obtain the perforated-patch configuration. To probe the K^+ selectivity of the transient outward current, 90 KCl and 30 K_2SO_4 were replaced with equimolar amounts of either NaCl and Na_2SO_4 or CsCl and Cs_2SO_4. The following drugs were used: nifedipine, TEA, 4-AP (Sigma), and (±)BAYK8644 (Alomone Labs). Pharmacological agents were dissolved daily in the appropriate solution at the concentrations stated, and were bath-applied by

gravity-fed superfusion at a flow rate of 2 ml/min, with complete solution exchange within the recording chamber in around 1 min. Experiments were carried out at room temperature (18–22°C).

Results

Transient Outward Currents and their Sensitivity to External Ca

Vascular smooth muscle cells display multiple types of transient and nontransient (sustained) outward currents, the latter including voltage-dependent (delayed rectifier) and large-conductance Ca^{2+}-dependent K^+ currents, as well as Ca^{2+}-dependent and -independent Cl^- currents (Nelson & Quayle, 1995). To isolate the fast transient outward K^+ current, we used conditions where the sustained conductances were minimized either with specific channel inhibitors or impermeant ions. We used TEA to suppress the BK current and reduce the delayed rectifier K^+ current. Preliminary experiments using a Cl^- -replete bathing solution revealed Cl^- currents. Almost complete replacement of Cl^- in the external solution with equimolar gluconate eliminated these currents. Under these experimental conditions, we recorded transient outward currents for depolarizing steps from high negative holding potentials (≤ −90 mV—required to remove the steady-state inactivation of this conductance).

Transient outward currents were evoked by depolarizing pulses ranging from −40 mV to +100 mV from a holding potential of −100 mV (Fig. 1*A*). The mean peak current-voltage (*I-V*) relationship obtained from six such experiments is shown in Fig. 1*B*. The transient outward K^+ current first activated at potentials positive to +20 mV, and had a peak amplitude of approximately 100 pA at +100 mV. The high activation threshold (> +20 mV) is a peculiar feature of this current, being remote from that reported for most transient outward K^+ currents in vascular smooth muscle cells (Beech & Bolton, 1989; James et al., 1995; but *see* Clapp & Gurney, 1991 and Smirnov & Aaronson, 1992).

Several fast transient K^+ currents in vascular smooth muscle cells have been reported to be modulated by external Ca, with peak current amplitude displaying an inverse dependence on Ca^{2+} concentration (i.e., decreasing external Ca^{2+} inceases the transient K^+ current (Beech & Bolton, 1989; Imaizumi, Muraki & Watanabe, 1990; Clapp & Gurney, 1991; Smirnov & Aaronson, 1992; Smirnov, Zholos & Shuba, 1992)). We tested the effects of altering external Ca. When the Ca^{2+} was lowered from 1 to 0.1 mM, the peak amplitude of the transient current elicited by a depolarizing pulse to +60 mV was increased substantially (Fig. 1*C*). Raising the external Ca^{2+} to 10 mM resulted in marked reduction of the current. Subsequent experiments were conducted using low external Ca^{2+} (0.1 mM), providing much larger transient outward currents (>3-fold in amplitude) than with that recorded in 1 mM Ca.

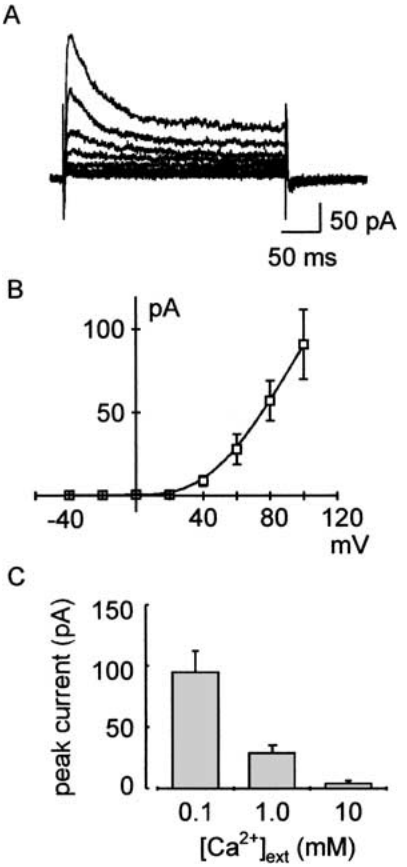

Fig. 1. Transient outward K^+ currents in saphenous myocytes. (*A*) A family of transient outward K^+ currents evoked by depolarizing potentials from −40 to +100 mV from a holding potential of −100 mV and recorded using the perforated-patch configuration, with external 1 mM Ca^{2+} and 10 mM TEA. Pipette and bath solutions contained 140 mM KCl and 140 mM NaCl as the main ions, respectively. (*B*) Peak current-voltage relationship recorded in these conditions. Each data point is the mean of measurements from six myocytes. The peak current amplitude was individually corrected for leakage current using suitably scaled hyperpolarizing pulses. A polynomial fit of the data is shown by the continuous line. (*C*) Histogram of the mean peak current evoked by a voltage step to +60 mV from a holding potential of −100 mV, in the presence of 0.1 (n = 4), 1 (n = 6) and 10 (n = 3) mM Ca^{2+} in the external, bathing, solution.

The Fast Transient Outward Current is not Affected by the A-Current Channel Blocker 4-AP

The fast transient outward current has a time course reminiscent of the A-type K^+ current, first described in neurons and then documented in many other cell types. The activation threshold of the current is, however, quite elevated and inconsistent with that

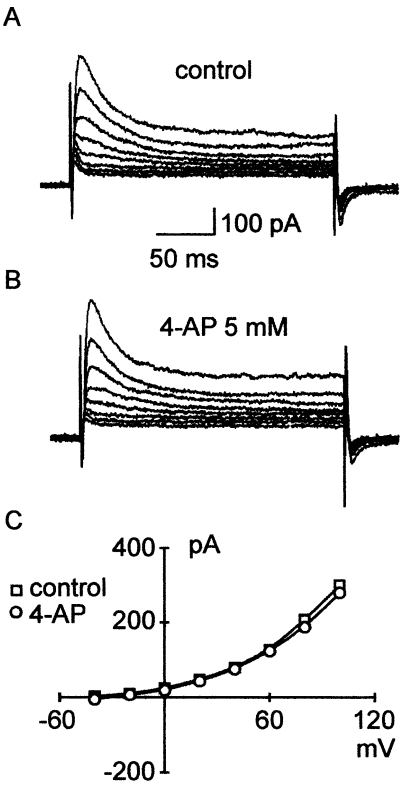

Fig. 2. Effects of 4-AP on the transient outward K^+ current. (*A*) and (*B*) Families of transient outward currents evoked by 20-mV depolarizing pulses from −40 mV to +100 mV (holding potential, −100 mV), in control conditions, and in presence of 5 mM 4-AP. The external solution contained 0.1 mM Ca^{2+} and 10 mM TEA. Pipette solution had 150 mM K^+ as the main cation. (*C*) Peak current-voltage relationships obtained for the recordings shown in *A* and *B*.

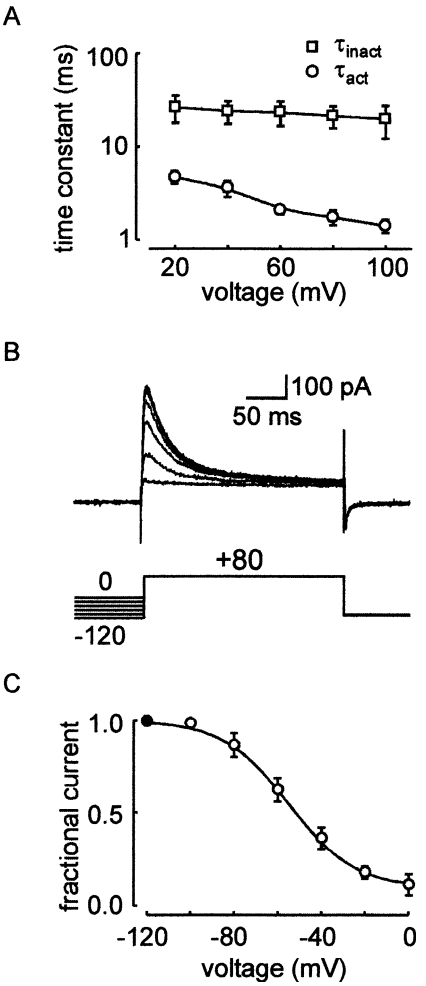

Fig. 3. Biophysical features of the transient outward K^+ current. (*A*) Voltage dependence of the time course of activation and inactivation of the transient outward K^+ current. The time constants were obtained by fitting the rise and decay phases of the transient K^+ currents at voltage steps to varying voltages with monoexponential functions. Data are presented for four myocytes recorded using the experimental conditions described in Fig. 2. (*B*) Family of transient outward K^+ currents evoked by a depolarizing pulse to +80 mV, following a 1-s conditioning prepulse to voltages from −120 mV to 0 mV. (*C*) Plot of peak outward K^+ current (normalized to the value recorded on stepping from −120 mV) as a function of the preconditioning voltage using data from five experiments similar to that shown in *B*. The experimental data were fitted with the equation: $I_v/I_{-120} = (1 - C)/(1 + \exp(zF(V - V_{1/2})/RT) + C$, where I_{-120} is the peak current recorded at +80 mV following a conditioning potential step to −120 mV. The parameters obtained were $C = 0.1$, $z = 1.7$, $V_{1/2} = -55$ mV.

reported for A-currents. To clarify the nature of this transient outward current we employed the A-current channel blocker 4-AP. This agent had no effect on the transient outward current. The results of this test are illustrated in Fig. 2, which presents a family of transient outward currents evoked by depolarizing pulses ranging from −40 mV to +100 mV from a holding potential of −100 mV, in control conditions and upon bath addition of 4-AP (5 mM). The lack of effect of 4-AP on the transient current is further illustrated by the *I-V* relationship obtained in absence and presence of 4-AP shown in Fig. 2. This result was confirmed in five similar experiments.

Biophysical Features of the Transient Outward Current

The general characteristics of the transient current recorded from saphenous myocytes with 150 mM K^+ in the pipette are shown in Fig. 3. The time course of both activation and inactivation of the current were well fitted by single-exponential functions. The time constants of these processes displayed differential voltage sensitivity. The activation rate was highly voltage-dependent (varying ~4-fold for an 80-mV change in membrane potential), whereas the inactivation time constant was only slightly voltage-dependent (Fig. 3*A*). The voltage dependence of the steady-state inactivation parameter of the transient outward current was determined by applying preconditioning pulses ranging from −120 to 0 mV for 1 s before stepping to a test potential of +80 mV

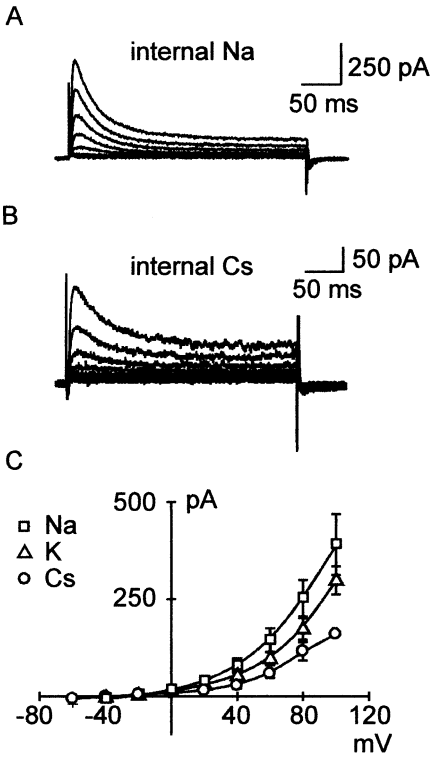

Fig. 4. Na^+ and Cs^+ ions can sustain the transient outward current. (*A*) and (*B*) Families of transient outward currents evoked by depolarizing pulses ranging from −60 to +100 mV (holding potential, −100 mV) recorded using pipette solutions containing either Na^+ or Cs^+ as the primary cation, and with external 0.1 mM Ca^{2+} and 10 mM TEA. Recordings were taken after complete ion exchange of the intracellular solution (8 and 11 min for the experiment in *A* and *B*, respectively), confirmed using the test described in the Methods section. (*C*) Mean peak current-voltage relationships obtained with Cs^+ (*circles*; $n = 3$), Na^+ (*squares*; $n = 3$), or K^+ (*triangles*; $n = 4$) in the pipette solution.

(Fig. 3*B*). The peak current as function of the conditioning voltage was well fitted by a Boltzmann function with $V_{1/2} = -55$ mV and $z = 1.7$, plus a constant (~0.1; *cf.* Fig. 3*C* legend). The need for a constant component could be due to either incomplete inactivation of the transient outward current, or to the presence of a small non-inactivating current.

Na^+ and Cs^+ Ions can Sustain the Transient Outward Current

To assess the selectivity of the channel carrying the transient outward current to monovalent cations, internal K^+ was completely replaced with either Na^+ or Cs^+. Both these cations can act as charge carriers; the peak current amplitude ratio of that recorded with internal K^+ was 1.30 for Na^+ and 0.55 for Cs^+ (Fig. 4). These results indicate that the ion channel sustaining the fast transient outward current in rat saphenous myocytes is nonselective, allowing Na^+ and Cs^+ to pass easily. A poor K^+ selectivity, a high activation threshold, and dependence on external Ca^{2+} are properties that do not, in so far as we are aware, match with any documented K^+ current. Instead, they resemble the characteristics of currents carried by monovalent cations passing through voltage-gated Ca^{2+} channels (Reuter & Scholz, 1977; Fenwick et al., 1982; Lee & Tsien, 1982; Almers et al., 1984; Lee & Tsien, 1984; Fukushima & Hagiwara, 1985).

We designed experiments to test this hypothesis, the results providing compelling evidence that the transient outward current in saphenous myocytes does, indeed, pass through Ca^{2+} channels.

The Inactivation Rates of the Inward Ba^{2+} Current and Transient Monovalent Outward Current Are Similar

Experiments showing that the transient outward current shares kinetic features with the Ca^{2+} current are shown in Fig. 5. The internal K^+ was replaced by Na^+ (140 mM) and the external solution contained 90 mM Ba. First, the myocte was depolarized to +80 mV, evoking a sizeable outward Na^+ current that displayed the typical kinetic features of the transient outward current. At different times the cell was then repolarized to −100 mV, to record inward Ba^{2+} tail currents that would pass through the voltage-dependent Ca^{2+} channels. The inactivation time course of the outward Na^+ current were compared with the instantaneous inward Ba^{2+} currents, estimated by monoexponential fits of the Ba^{2+} tail currents (Fig. 5*B*). The good match between the inactivation rate of the outward Na^+ current and the decay of the instantaneous inward Ba^{2+} tail currents suggests that the ion channel underlying the outward Na^+ and inward Ba^{2+} currents is the same. Similar results were obtained in three experiments. These data underpin the view that the transient outward current is sustained by a monovalent cation flux through voltage-gated Ca^{2+} channels.

The Transient Outward Monovalent Current Is Affected by Ca^{2+} Channel Modulators

Arguably, a more direct test of the hypothesis that the transient monovalent current goes through Ca^{2+} channels is to probe the action of Ca^{2+} channel modulators on the current. The most common voltage-gated Ca^{2+} channel expressed in arterial smooth muscle cells belongs to the high voltage-activated L-type Ca^{2+} channel family, which is effectively blocked by dihydropyridines such as nifedipine. Nifedipine (10 μM) exerts a marked inhibition of the transient outward Na^+ current (evoked by a depolarization to +80 mV from a holding potential of

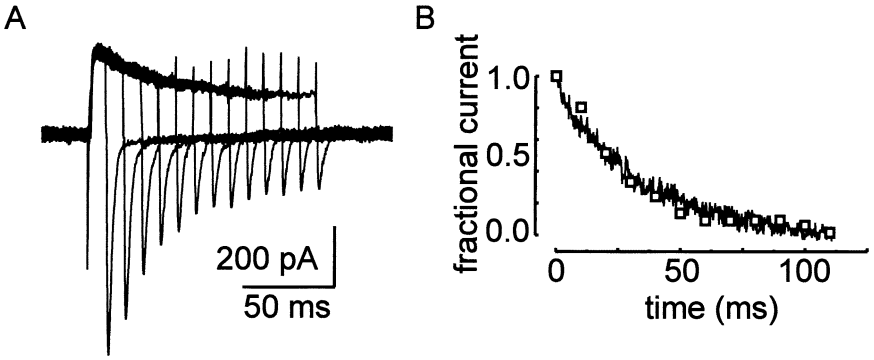

Fig. 5. Inactivation features of the Ca^{2+} and the nonselective transient outward currents. (*A*) Current traces obtained from a myocyte using internal 140 mM Na^+ and external 90 mM Ba^{2+} solutions. A variable-duration prepulse to +80 mV preceded a constant test pulse to −100 mV. The time course of inactivation of the Ba^{2+} current at +80 mV was determined from the envelope of the peak tail currents recorded at −100 mV. (*B*) Plot showing the decay of the transient outward Na^+ current and the instantaneous inward Ba^{2+} (tail) currents (*squares*) for the cell depicted in panel *A*. The instantaneous inward currents were estimated by fitting the Ba^{2+} tail current decays with monoexponential functions, extrapolating the value to time zero. To afford comparison, current values for both conditions were normalized, after subtraction of the steady-state component.

−100 mV, Fig. 6*A*). The inward Ba^{2+} current, evoked by a depolarizing pulse to −10 mV from the same holding potential in 90 mM external Ba, was similarly inhibited by 10 μM nifedipine (Fig. 6*A*). The mean current block (expressed as fractional residual current) exerted by 10 μM nifedipine on the transient outward Na^+ current and on the inward Ba^{2+} current was similar, being 0.22 ± 0.08 and 0.27 ± 0.11, respectively ($n = 3$; Fig. 6*C*). These results support the view that the transient outward monovalent current we recorded from rat saphenous arterial myocytes is conducted by voltage-dependent dihydropyridine-sensitive Ca^{2+} channels.

The results do not, however, provide conclusive proof of the involvement of Ca^{2+} channels, as other outward currents can be inhibited by nifedipine, as well as by other organic Ca^{2+} channel blockers (Erdelyi, 1987; Lin, Wang & Fedida, 2001). To address this point, we used the specific Ca^{2+} channel agonist BAYK8644. Figure 6*B* illustrates typical recordings in response to a depolarizing step to +80 mV, from a holding potential of −100, in control conditions and after bath application of BAYK8644. The Ca^{2+} channel agonist increased the transient outward current by ~30%, without affecting its time course. The bar histogram of Fig. 6*C* summarizes the effect of BAYK8644 on the peak transient current amplitude from three experiments.

Discussion

Here we report the results of investigations characterizing a fast transient outward current recorded from isolated myocytes of rat saphenous artery using the perforated-patch configuration, and demonstrate that this current flows through Ca^{2+} channels. The following lines of evidence support this view. First, both Na^+ and Cs^+, virtually impermeant ions through K^+ channels, were capable of carrying appreciable currents (Fig. 4). Moreover, Na^+ seemed to conduct more current and Cs^+ less current than K^+, as reported for monovalent cation currents passing through Ca^{2+} channels in other tissues (Fukushima & Hagiwara, 1985). Second, the decay phase of the transient outward monovalent current matches the decay of the instantaneous inward Ba^{2+} tail current through Ca^{2+} channels. That is, the envelope of the tail current amplitude from Ca^{2+} channels mirrored exactly the decay phase of the transient outward monovalent current taken at the same potential. Third, the transient outward monovalent current was inhibited by nifedipine and enhanced by BAYK8644, a specific blocker and activator of the L-type Ca^{2+} channel, respectively. In particular, nifedipine inhibited the transient outward monovalent current, and the inward Ba^{2+} current to the same extent (Fig. 6). Additional evidence that the transient outward current is sustained by Ca^{2+} channels is provided by our previous work on this preparation (Catacuzzeno et al., 2000). This current was never observed when the external solution contained the nonspecific Ca^{2+} channel blocker Cd^{2+}. The $V_{1/2}$ for steady-state inactivation assessed for the outward monovalent current of rat saphenous myocytes (−55 mV) is distinct, being about 20 mV more hyperpolarized than that for Ca^{2+} currents in smooth muscle cells (−25/−30 mV, Smirnov & Aaronson 1992). This inconsistency is likely due to the different ionic conditions used in these studies. Given the marked screening effects observed on the gating properties of smooth muscle Ca^{2+} currents (Aaronson et al., 1988; Campbell et al., 1988; Lang, 1990), we would predict (at low external divalent ion concentration) a shift in the inactivation range towards more hyperpolarized potentials. In contrast, the voltage dependence of steady-state inactivation should not be significantly affected by screening effects, and indeed was close to the values reported for Ca^{2+} currents in other smooth muscle cells (Aaronson et al., 1988; Lang 1990).

Modelling studies of inward currents through Ca^{2+} channels in skeletal muscle fibers have generally

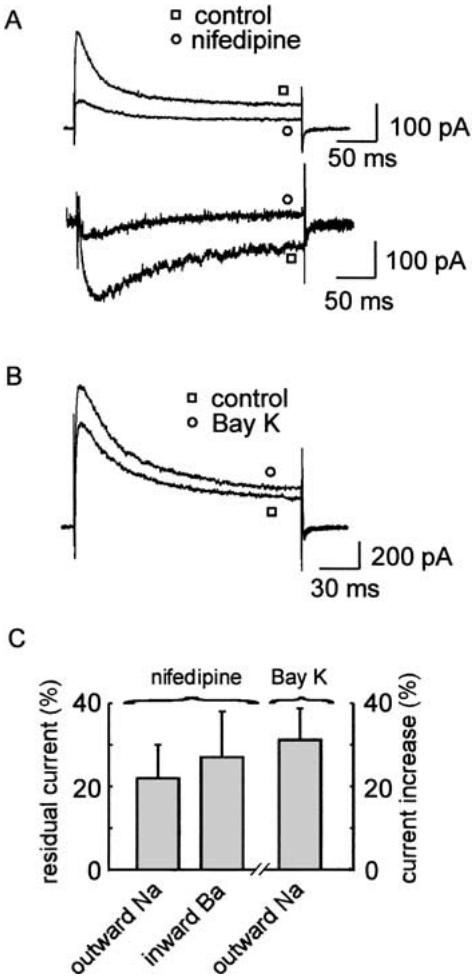

Fig. 6. Pharmacological evidence that the transient outward current goes through Ca^{2+} channels. (*A*, *top*) Transient outward Na^+ currents (140 mM Na^+ in the pipette), evoked by a constant depolarizing test pulse to +80 mV (holding potential −100 mV) in control conditions and after superfusion with 10 μM nifedipine. The external solution contained 0.1 mM Ca^{2+} and 10 mM TEA. The fractional residual peak current after application of nifedipine was 0.17. (*A*, *bottom*) Inward Ba^{2+} currents (90 mM Ba^{2+} in the external solution and 140 mM Cs^+ in the pipette), evoked by a constant depolarizing pulse to +10 mV (holding potential −100 mV), in control conditions and after superfusion of 10 μM nifedipine. The fractional residual peak current after addition of nifedipine was 0.23. (*B*) Transient outward Na^+ currents (140 mM Na^+ in the pipette) evoked by a constant depolarizing pulse to +80 mV (holding potential, −100 mV), in control conditions and after perfusion with 1 μM BAYK8644. The external solution contained 0.1 mM Ca^{2+} and 10 mM TEA. (*C*) Histogram showing the inhibitory effect of 10 μM nifedipine on the outward Na^+ ($n = 3$), and inward Ba^{2+} currents ($n = 3$), and the activating effect of 1μM BAYK8644 on the outward Na^+ current ($n = 2$).

assumed that Ca^{2+} channels have two binding sites with high affinity for Ca^{2+} (Almers & McCleskey, 1984; Almers et al., 1984b), making the channels very selective. When external Ca^{2+} is above 10 μM, at least one site is occupied by the divalent, and monovalent cations cannot pass through the channel. When neither of the binding sites is occupied, which occurs at negative voltages when external Ca^{2+} is extremely low (<10 μM), monovalent cations can pass easily, and can carry even more current than Ca^{2+}.

We have shown that in rat saphenous myocytes, monovalent cations can pass outward through Ca^{2+} channels, when the external Ca^{2+} is as high as 0.1–1 mM (internal Ca^{2+} being in the physiological range, i.e., 100–200 nM). With no imposed voltage, this Ca^{2+} concentration would be sufficient to leave virtually no Ca^{2+} channel unoccupied, and thus prevent monovalent flux. One way to reconcile our data with the Almers and McCleskey model is that the outward monovalent flow through Ca^{2+} channels occurs because of a voltage-dependent expulsion of Ca^{2+} from the Ca^{2+} binding site, making the channel permeable to Na^+ and other monovalent cations. In accordance with this view, the current is first evoked at voltages some 60–80 mV positive to the activation threshold for Ca^{2+} channels (Nelson & Quayle, 1995), and increases when external ("blocking") Ca^{2+} ion concentration is reduced.

More recently, a mean-field theory of Ca^{2+} channel selectivity has been developed (Nonner et al., 1999, 2000) that predicts the observed increase in Na^+ permeation through Ca^{2+} channels under low Ca^{2+} conditions. The theory quantitatively shows that when external Ca^{2+} is lowered to submicromolar concentrations, Na^+ ions access the Ca^{2+} channel's selectivity filter, driven by the unsatisfied electroneutrality requirement around the negatively charged carboxylate groups of the EEEE locus (Ellinor et al., 1995). Under these conditions Na^+ ions can overcome the strong repulsive forces due to volume exclusion, forces that under more physiological conditions keep Na^+ ions out of the filter and endows the channel with a high selectivity for divalent versus monovalent cations.

The Ca^{2+} Channel Type Sustaining the Transient Outward Current

Voltage-dependent Ca^{2+} currents have been observed in almost all types of arterial myocytes (Nelson et al., 1990). Their kinetics, voltage dependence and pharmacological profile underpins the proposal that two major classes of Ca^{2+} channels are found in these cells (Bean et al., 1986; Loirand et al., 1986; Benham, Hess & Tsien, 1987; Yatani et al., 1987; Aaronson et al., 1988). The dominant voltage-dependent Ca^{2+} channel in arterial myocytes activates at rather high voltages (*ca* −10 mV), inactivates slowly, and is inhibited by dihydropyridines (exemplified by nifedipine). This channel has been referred to as the L-type Ca^{2+} channel. Dihydropyridine-insensitive, rapidly inactivating Ca^{2+} channels with much lower activation threshold (T-type Ca^{2+} channels) have been reported in some arterial myo-

cytes (Bolton et al., 1988). The sensitivity to dihydropyridines is a cardinal feature, as only the L-type Ca^{2+} channels, but not the T-type or any of the other types of Ca^{2+} channels that have now been described in vascular and nonvascular tissues (i.e., N, P/Q, R) contain the high-affinity stereospecific binding sites for this class of compounds (Hille, 1992). This makes nifedipine and other dihydropyridines such as BAYK8644 unique pharmacological tools to identify the L-type Ca^{2+} channel. This notion and the observation that Ba^{2+} currents as well as the transient outward monovalent currents were both shown to be sensitive to the dihydropyridines nifedipine and BAYK8644, identify the Ca^{2+} channel responsible for carrying monovalent cations as being of the L-type. Moreover, the outward current appears to be conducted by a homogeneous population of L-type Ca^{2+} channels, and it is unnecessary to invoke the participation of other types of Ca^{2+} channel.

Transient Outward Currents

Transient outward currents have been reported in many vascular myocytes (Beech & Bolton, 1989; Hume & Leblanc, 1989; Imaizumi et al., 1990; Clapp & Gurney, 1991; Smirnov & Aaronson, 1992; James et al., 1995). In these reports, the impermeability of the channel to monovalent cations other than K^+, and its inhibition by 4-AP, were taken as evidence that they were A-currents. In no instance were the currents identified as a flux of K^+ through Ca^{2+} channels. The sensitivity to external Ca^{2+} that was observed in some cases, with Ca^{2+} significantly reducing the current amplitude and shifting the steady-state inactivation curve to the left, was taken as a result of the screening effect of external Ca^{2+} (*see* Beech & Bolton, 1989; Smirnov & Aaronson, 1992).

In the present study we show that, unlike most vascular smooth muscle cells, saphenous myocytes do not express an A-current (either true A or A-like). We exclude the presence of even a minor A-current component in this tissue for the following reasons: 1) A-currents have activation thresholds typically near −40 mV, but no outward current could be evoked even at voltages positive to this value. The transient outward K^+ current first activates at voltages above +20 mV. 2) 4-AP has no effect on the transient outward K^+ current.

It can be finally added that consistent with this conclusion, the decay phase of the high-voltage-activated transient outward K^+ currents was always well fitted by single-exponential functions, consistent with a homogeneous channel population. Given the proposed modulatory role of the excitability of vascular myocytes assigned to these currents (i.e., 4-AP-sensitive currents were shown to suppress the action potential in guinea pig aorta and human mesenteric artery [Hara, Kitamura & Kuriyama, 1980; Smirnov & Aaronson, 1992]), the lack of the A-current in saphenous myocytes may underline a distinct electrophysiological profile of these cells.

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